Format of the Amendments

The specific changes to the specification are shown on a separate set of pages attached hereto and entitled VERSION WITH MARKINGS TO SHOW CHANGES MADE, which follows the signature page of this Amendment. On this set of pages, the insertions are underlined while the **[deletions are in brackets and bolded]**.

Claim Objections and Rejections

Claims 1-11, 13, 16-30, 32 and 36-55 are currently pending in this application. Claims 1, 11, 19, 30, 51, 52 and 55 are objected to because they allegedly recite and/or encompass nonelected species. Claims 52-54 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter not disclosed in the specification. Claims 2, 17, 18, 20, 37 and 38 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly failing to particularly point out and distinctly claim the subject matter the applicant regards as the invention. Claims 52 and 53 have been rejected under 35 U.S.C. § 102(b) as being anticipated by the Pierce Catalog and Handbook, 1994-95. Claims 1-11, 13, 16-30, 32 and 36-55 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over De La Fuente *et al.*, Hastie *et al.*, and Rothschild *et al.* and the Pierce Catalog and Handbook, 1994-95. Applicants respectfully traverse each of the above objections and rejections for the reasons set forth below.

Claim Objections

The Examiner objects to claims 1, 11, 19, 30, 51, 52 and 55 as allegedly reciting and/or encompassing non-elected species. Amendment of each of these claims to include only the elected species is requested.

Applicants respectfully submit that claim 55, which was added in response to the Restriction Requirement of July 5, 2001 (Paper No. 10), recites the elected species sulfosuccinimidyl-2-(biotinamido)ethyl-1,3-dithiopropionate (see Amendment submitted February 22, 2002). Additionally, Applicants respectfully submit that claims 1, 11, 19, 30, 51 and 52 need not be amended to recite the single elected species at this time. According to M.P.E.P. § 809.02(a), claims that are subject to a species election will only be restricted to the elected species if no generic claim is found allowable. Upon finding a generic claim allowable, Applicants are entitled to consideration of claims to additional species.

In light of the foregoing, Applicants maintain that amendment of Claims 1, 11, 19, 30, 51 and 52 to include only the elected species is not presently required. Applicants respectfully request that the Examiner withdraw these claim objections.

Rejections Based on 35 U.S.C. § 112, first paragraph

Claims 52-54 are rejected under 35 U.S.C. § 112, first paragraph as being inadequately described in the specification. The Examiner asserts that the specification fails to describe components of the claimed kit other than the cell membrane impermeable reagent. Furthermore, the Examiner asserts that the specification does not adequately describe the information contained in the 'printed matter' with sufficient particularity to show that Applicants had possession of the claimed kit at the time of filing the instant application.

Applicants respectfully submit that the specification provides sufficient disclosure of specific kit components (e.g. a suitable buffer) and the contents of the printed matter which instructs in the use of the claimed kits. In particular, the Examiner's attention is drawn to pages 20-21, bridging paragraph, where it is stated that kits may contain "suitable buffers for administration (perfusion) to intact organs, tissues or animals" and that the printed matter may contain "instructions for practicing the methods of the invention, as set forth herein." According to this paragraph, buffers suitable for perfusion, for example those described throughout the specification, can be included with claimed kit. Likewise, the printed matter can contain, for example, printed methods for using the cell membrane impermeable reagent that are described throughout the instant application.

Example 1 of the instant application specifically describes perfusion buffers that are suitable for use with the cell membrane impermeable reagent. For example, page 22, lines 1-5 describe the use of borate-buffered saline at pH 9.0 as an example of a perfusion buffer. Additionally, the example states that similar buffers having a pH ranging from 7.5 to 9.5 may be used.

Example 1 also describes methods for administering the cell membrane impermeable reagent into a perfusible space. For example, page 21, line 30 to page 23, line 9 describes a method that can be set forth as printed matter which instructs in the use of the cell membrane impermeable reagent for administration into luminal spaces. This method also instructs in the subsequent purification of molecules bound by the cell membrane impermeable reagent. For

example, the use of immobilized streptavidin in the purification of molecules bound by a biotin-containing cell membrane impermeable reagent is specifically taught.

In light of the foregoing, Applicants maintain that claims 52-54 are adequately described by the specification as required by 35 U.S.C. § 112, first paragraph. Accordingly, Applicants respectfully request that the Examiner withdraw this rejection.

Claim Rejections Based on 35 U.S.C. § 112, second paragraph

Claims 2, 17, 18, 20, 37 and 38 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The Examiner asserts that the claims fail to set forth how a reagent which non-specifically binds to any molecule can identify "an organ-specific or a tissue-specific molecule" (as recited in claims 2 and 20), "a polypeptide" (as recited in claims 17 and 37) or "a lipid or a carbohydrate" (as recited in claims 18 and 38). The Examiner requests clarification.

Applicants respectfully submit that claims 2, 17, 18, 20, 37, and 38 are clear as written. The test for whether a claim limitation is definite turns on the way one skilled in the art would understand that limitation in view of the specification. *ATMEL Corp. v. Information Storage Devices, Inc.*, 198 F.3d 1374, 1378, (Fed. Cir. 1999) citing *In re Dossel*, 115 F.3d 942 (Fed. Cir. 1997). In each of the rejected claims, the cell membrane impermeable reagent is introduced into a perfusible space and allowed to bind to molecules exposed on the luminal surface of the cells that line the perfusible space. The cell membrane impermeable reagent does not have a substantial preference for the type of molecule that is bound. Claims 17 and 37 point out that one type of molecule that may be bound by the cell impermeable reagent is a polypeptide. Claims 18 and 38 state that other types of molecules may also be bound by the cell impermeable reagent such as lipids or carbohydrates. Claims 2 and 20 simply state that the bound molecules may be those ones that are found only in certain organ(s) or tissue(s).

It is noted that the purpose of the claim is to legally define the metes and bounds of the property right, not to provide a production specification. The invention is to be enabled by the specification, not the claims. Applicants respectfully submit that a person of ordinary skill in the art can readily ascertain whether any particular molecule in question can be non-specifically bound by the cell membrane impermeable reagent in question.

In light of the foregoing, Applicants maintain that claims 2, 17, 18, 20, 37 and 38 are not indefinite. Accordingly, withdrawal of this rejection is respectfully requested.

Claim Rejections Under 35 U.S.C. § 102(b)

Claims 52 and 53 are rejected under 35 U.S.C. § 102(b). In particular, the Examiner asserts that the Pierce Catalog & Handbook, 1994-95 anticipates claims 52 and 53 because it allegedly teaches "both reagents and kits, and the general guidance and instruction for use of each of these in the purification of a protein present on the surface of a cell." The Examiner notes, however, that "claim 54 is not included in the basis of this rejection because it indicates that the printed material specifically instructs for the administration into a lumen which the Pierce Catalog & Handbook does not specifically teach."

Applicants respectfully submit that claims 52 and 53 are not anticipated by the Pierce Catalog & Handbook because they also recite that limitation that the printed material instructs for the administration into a lumen. The Examiners attention is drawn to claim 52 from which claim 53 depends. Specifically, claim 52 recites, in relevant part, that the kit comprises "printed matter instructing use of the cell membrane impermeable reagent for administration into a lumen of an intact organ or an intact animal to react the cell membrane impermeable reagent with a molecule expressed on the luminal surface to isolate the reagent-reacted molecule."

In light of the foregoing, Applicants maintain that both claim 52 and dependent claim 53 are free of the teachings of the Pierce Catalog & Handbook and therefore, are not anticipated under 35 U.S.C. § 102(b). Accordingly, withdrawal of this rejection is respectfully requested.

Claim Rejections Under 35 U.S.C. § 103(a)

Claims 1-11, 13, 16-30, 32 and 36-55 are rejected under 35 U.S.C. § 103(a). The Examiner asserts that De La Fuente *et al.*, Hastie *et al.*, or Rothschild *et al.* in combination with the Pierce Catalog & Handbook render the claimed subject matter obvious. In particular, the Examiner asserts that De La Fuente *et al.* teach the use of noncleavable NHS-LC-biotin for the identification and isolation of molecules which are specific to perfusible spaces within rat pulmonary tissue. Hastie *et al.* allegedly teach the use of noncleavable NHS-LC-biotin for the identification of components in cow tracheae. Rothschild *et al.* (U.S. Patent No. 5,948,624) allegedly provide a general description of heterobifunctional crosslinkers for use in detection and

isolation of biomolecules. The Examiner then states that none of these references specifically teach the use of a cleavable crosslinker. The Examiner asserts that the Pierce Catalog & Handbook provides this element by allegedly teaching the use of the cleavable crosslinker NHS-SS-biotin for the isolation or detection of a molecule of interest. The Examiner asserts that one skilled in the art would be motivated to combine the teachings of De La Fuente *et al.*, Hastie *et al.*, or Rothschild *et al.* with the Pierce Catalog & Handbook to produce the claimed invention because they would be motivated to remove biotin from isolated proteins. A reasonable expectation of success allegedly arises from the general teachings of the art at the time of the invention.

Applicants respectfully submit that claims 1-11, 13, 16-30, 32 and 36-55 are not obvious in light of the art cited by the Examiner. For sake of clarity Applicants will provide remarks to each individual combination of references separately as follows.

The combination of Rothschild *et al.* and the Pierce Catalog & Handbook

Rothschild *et al.* describe the use of a variety of reagents to detect and isolate target components from mixtures such as biological samples. A careful reading of Rothschild *et al.* reveals that none of the samples taught therein include luminal or other perfusible spaces of organs or tissues. As such, Rothschild *et al.* fails to teach administering the membrane impermeable reagent into a perfusible space in an intact organ or an intact animal as recited in claims 1-11, 13, 16-30, 32 and 36-55.

The combination of Rothschild *et al.* with that of the Pierce Catalog & Handbook fails to teach all of the elements of claims 1-11, 13, 16-30, 32 and 36-55. The Pierce Catalog & Handbook fails to teach administration of NHS-SS-biotin (or any other crosslinking or affinity reagents) into a perfusible space in an intact organ or an intact animal. Thus, even in combination, Rothschild *et al.* and the Pierce Catalog & Handbook fail to teach every element of the claimed invention.

In light of the foregoing, Applicants maintain the claims 1-11, 13, 16-30, 32 and 36-55 are not obvious over the combination of Rothschild *et al.* and the Pierce Catalog & Handbook. Accordingly, Applicants respectfully request withdrawal of this rejection.

The combination of Hastie *et al.* and the Pierce Catalog & Handbook

Hastie *et al.* describe the use of the noncleavable NHS-LC-biotin reagent to label respiratory tract cilliary membranes found in cow tracheae. The tracheae the were used in the

experiment by Hastie *et al.* were previously removed from the cows and cut into halves. This prepared tissue was then incubated with the NHS-LC-biotin reagent. Accordingly, Hastie *et al.* fail to teach administering the membrane impermeable reagent into a perfusible space in an intact organ or an intact animal as recited in claims 1-11, 13, 16-30, 32 and 36-55.

The combination of Hastie *et al.* with that of the Pierce Catalog & Handbook fails to teach all of the elements of claims 1-11, 13, 16-30, 32 and 36-55. The Pierce Catalog & Handbook fails to teach administration of NHS-SS-biotin (or any other crosslinking or affinity reagents) into a perfusible space in an intact organ or an intact animal. Thus, even in combination, Hastie *et al.* and the Pierce Catalog & Handbook fail to teach every element of the claimed invention.

In light of the foregoing, Applicants maintain the claims 1-11, 13, 16-30, 32 and 36-55 are not obvious over the combination of Hastie *et al.* and the Pierce Catalog & Handbook. Accordingly, Applicants respectfully request withdrawal of this rejection.

The combination of De La Fuente *et al.* and the Pierce Catalog & Handbook

De La Fuente *et al.* teach the introduction of the noncleavable reagent NHS-LC-biotin into the vasculature of isolated rat lung. The lung tissue is then homogenized and a membrane fraction is isolated. The membrane fraction is analyzed by identifying the biotin-labeled proteins which have been electrophoresed, transferred to nitrocellulose then detected using a commercial biotin detection reagent. De La Fuente *et al.* do not teach labeling of vasculature or other perfusible tissue with cleavable biotin-containing reagents such as NHS-SS-biotin. Furthermore, De La Fuente *et al.* do not teach the isolation of proteins using cleavable biotin-containing reagents, such as NHS-SS-biotin. In fact, De La Fuente *et al.* specifically teach the use of lectin affinity chromatography to isolate the labeled proteins which suggests that a cleavable reagent would not be an appropriate choice for the isolation of proteins from complex tissues such as intact blood vessels.

There is no motivation to combine the teachings of De La Fuente *et al.* with the teachings of the Pierce Catalog & Handbook to obtain the subject matter of claims 1-11, 13 and 16-18. The Pierce Catalog & Handbook teaches the use of cleavable NHS-SS-biotin for isolating proteins from a mixture of proteins but fails to teach the administration of any biotinylated reagent (cleavable or noncleavable) into a perfusible space in an intact organ or an intact animal. One of ordinary skill in the art would not be motivated to use cleavable crosslinkers or cleavable affinity reagents in the labeling of molecules accessible in a perfusible space of a tissue as recited in claims 1-11, 13

and 16-18, because of the potential for premature cleavage. Such premature cleavage would result in partial or even complete loss of label and thus erroneous results. Thus, a skilled artisan would seek to use most stable available reagent for the purpose of labeling. Accordingly, there is no motivation to combine the use of noncleavable reagents as taught by the Pierce Catalog Handbook with the teachings of De La Fuente for the purpose of labeling molecules lining a perfusible space.

Additionally, there is neither motivation to combine the teachings of De La Fuente *et al.* with the teachings of the Pierce Catalog & Handbook to obtain the subject matter of claims 19-30, 32 and 36-55 nor is there a reasonable expectation that such a combination would be successful. The Examiner's attention is drawn to the fact that cleavable reagents have been known since at least 1995 as demonstrated by the publication date of the Pierce Catalog & Handbook. Furthermore, the De La Fuente *et al.* reference was published in early 1997 in the American Journal of Physiology, a widely read scientific and medical journal. Applicants did not file a United States Patent Application claiming the use of cleavable reagents in the isolation of molecules lining perfusible spaces until March 20, 2000. The Examiner cites no reference that was published during the three years between the publication of De La Fuente *et al.* and the filing of the instant patent application which shows this combination. Such a publication is absent because there was neither motivation to combine the teachings of De La Fuente *et al.* with the teachings of the Pierce Catalog & Handbook nor was there a reasonable expectation of success.

For example, one of ordinary skill in the art would not reasonably expect that a cleavable reagent could be effectively used for the isolation of molecules from the surface of cells lining a perfusible space of an intact organ or an intact animal. Cleavable reagents such as NHS-SS-biotin are most useful in situations where the environment can be easily controlled so as to prevent accidental or premature cleavage of the reagent. One of ordinary skill in the art would not reasonably expect that introduction of a cleavable reagent into an environment where the researcher has much less control over the reaction conditions, such as in the perfusion of tissues or organs within an intact animal, would provide a successful method for the consistent and nonspecific isolation of molecules lining the perfusible space. The use of cleavable reagents in less complicated systems, such as in vitro tissue culture, provides no suggestion that the use of such cleavable reagents would be successful in complex mixtures such as a whole tissue homogenate. Thus, one of ordinary skill in the art would not be motivated to use cleavable reagents for this purpose nor would they reasonably expect success from such a combination.

Accordingly, there exists no motivation to combine the teachings of De La Fuente with those of the Pierce Catalog & Handbook nor is there a reasonable expectation that such a combination would be successful.

In light of the foregoing, Applicants maintain the claims 1-11, 13 and 16-18 are not obvious over the combination of De La Fuente *et al.* and the Pierce Catalog & Handbook. Accordingly, Applicants respectfully request withdrawal of this rejection.

CONCLUSION

For the foregoing reasons, it is respectfully submitted that the rejections set forth in the outstanding Office Action are inapplicable to the present claims. Accordingly, Applicants request the expeditious allowance of the pending claims.

The undersigned has made a good faith effort to respond to all of the rejections in the case and to place the claims in condition for immediate allowance. Nevertheless, if any undeveloped issues remain or if any issues require clarification, the Examiner is respectfully requested to call the undersigned to discuss such issues.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 9/6/02

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

Insertion on page 1, line 1

This application claims priority to U.S. Provisional Patent Application No. 60/139,579, filed June 17, 1999, now abandoned.

Please replace the paragraph that begins on page 9, line 22 with the following paragraph:

[Figure 3 (upper panel)] Figure 3A shows a representation of the results of a Western blot of a PAGE separating vascular lumen-exposed polypeptides, prepared by the methods of the invention, stained with an antibody that recognizes a polypeptide that is only expressed on the lumen of vascular endothelial cells (PECAM-1) and an antibody that recognizes a polypeptide only expressed intracellularly (the Golgi 58 kDa polypeptide), as described in Example 1, below. **[Figure 3 lower panel]** Figure 3B shows a Western blot of total tissue homogenate stained with anti-Golgi 58 kDa polypeptide antibody.

Replacement of paragraph beginning on page 14, line 20

The first domain of the cell membrane impermeable reagent comprises a chemical moiety capable of covalently and non-specifically binding to a molecule expressed on the luminal surface of a cell lining a perfusible space *in situ* or *in vivo*. The moiety can be reactive to, e.g., amine, carboxyl, carbohydrate or sulfhydryl groups on the luminally-expressed molecule. The chemistry and reagents for such reactions are well known in the art; see, e.g., catalog of Pierce Chemicals (Rockville, IL); **[http://www.piercenet.com/Products/]** www.piercenet.com.